ON THE LIGHT ORGAN OF FIRE-FLIES.

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Some of the most interesting problems connected with the light of the fire-fly belong to physics and chemistry rather than to biology. These are the problems connected with the nature and source of the light. The physical properties have been studied by Dubois, Langley, Very, Young, Watosé, and others, with essentially the same results. The spectrum of the light given by the Lampyridae is perfectly continuous, without any trace of lines, either dark or bright. It lies almost wholly within that portion of the spectrum which is visible to the human eye, although the presence of actinic rays has been demonstrated by photography.

The most remarkable fact about the light is that it is practically, if not absolutely, a " light without heat ". In 1902 the late Professor Langley, of Washington, told me that after thorough experiment with the most delicate thermal apparatus known to science he could authorize the statement that not more than one-thousandth, if any at all, of the energy expended in the flash of the fire-fly is lost in heat. When one considers that with our ordinary artificial lights less than one per cent. of the energy is sometimes available for light, and even with the electric arc only ten per cent., the wonderful economy of this " cheapest form of light " becomes very apparent.

Were it not for the small size of the light organs, and the minute quantity of photogenic substance obtainable for analysis, doubtless more would long since have been done by the chemist toward determining its nature. If this substance could be analysed, produced artifically, and used for lighting purposes conveniently, its economic importance could scarcely be estimated.

While organic light has always been a source of interest to scientists, little has been done beyond the investigation of its physical properties. The generally accepted theory as to the origin of the light is that it results from the oxidation of some substance secreted by the cells of the light organ. The purpose of my investigation, begun at Cornell University in June, 1901, has been to study the structure of the light organs with the hope that the knowledge gained would be of value in determining the nature of the photogenic process. In other words, I wished to see whether the tracheal structures were such as to presuppose some special demand for oxygen in that portion of the body.

In spite of the number of species and wide distribuiton of fire-flies in America, I found that practically no histological work had ever been attempted. The very limited literature on the entomological side of the question was in German and French, and was confined to a very few writers. Comparisons of structure were, therefore, possible only with two European species, *Lampyris splendidula*, studied by Max Schultze in 1864, by Wielowiejski in 1882, and by Bongardt in 1903; and *Luciola italica*, studied by Emery in 1884.

The species chosen for my work was *Photinus marginellus*, a small, light brown fire-fly very common in the vicinity of Cornell University during June and July. Of several hundred insects collected during three summers, only a small number were females. The female, although having fitly developed elytra and wings, apparently does not fly, as I have found all those collected either on the ground or on some low plant near the ground.

The female has a single somewhat spherical light organ in the centre of the fifth abdominal segment, and the light is much inferior to that of the male.

The larva of *Photinus marginellus* has never been recorded, and I was never able to find it. Neither did I ever succeed in getting eggs to develop, although I made several attempts.

All my work was confined to light organs of the adult male. These are in the form of two plates, completely filling the ventral part of the fifth and sixth abdominal segments. They lie directly upon the very thin hypodermis. The underlying cuticle is transparent, allowing free emission of the light. The main tracheae of the photogenic segments send numerous branches into the light organs, perforating the whole dorsal surface.

Where a section of the light organs of *Photinus marginellus* is studied with the microscope, it is found to be composed of two clearly defined layers. The ventral layer is composed of fairly regular, polygonal cells filled with a dense content of coarse granules. With transmitted light these granules are a dull brown. With reflected light, in both fresh material and prepared sections, they show a peculiar chalky whiteness which has given to the dorsal layer the name of "chalky layer".

In 1857 Kölliker analysed the granules from the dorsal layer of *Lampyris* and found them to be crystals of urate salts. This analysis has been verified by recent workers, among them Bongardt, in 1903. Granules, identical in appearance, are to be found in the fat body in the photogenic segments of *Photinus*.

The ventral layer differs widely in structure from the dorsal. The tracheae continue to branch even more profusely in an arborescent manner characteristic of the photogenic tissue. There is a striking feature of the tracheal structures in the ventral layer which is wholly different from that in the dorsal layer. Each of the larger branches coming through the dorsal layer is approximately vertical in its course. Each of these tracheal trunks with its branches is surrounded by a cylindrical mass of tissue which is transparent in fresh material. These cylinders are distributed through the tissue with considerable regularity. Between these are cells very irregular in size and shape. These cells are called the "parenchyma cells".

Near the periphery of the cylinders the small tracheal branches divide, sending out generally two, rarely three or four, tracheoles. These tracheoles are fine tracheal branches characterized by having no spiral thickening of the intima. When fresh material, treated for half an hour with .5 per cent. osmic acid, then for a few minutes with weak caustic potash, is placed under the microscope ventral side up, the tracheae can readily be traced. The outlines of the cylinders persist, the branching of the tracheae is seen, and the origin and course of the tracheoles. In all such preparations studied the tracheoles from different cylinders anastomosed, forming a close tracheolar network throughout the area of parenchyma. Although the tracheoles are generally cut in sections parallel to the axis of the cylinder, yet even here occasional demonstrations of anastomosing are to be found. I have found no indication of the penetration of the parenchyma cells by the tracheoles; they appear always to be intercellular in their course.

Wielowiejski found anastomosing of the tracheoles to be the rule in *Lampyris splendidula,* although he admits of some exceptions. Wistinghausen and Holmgren, in their studies of tracheal-ending in the silk glands of caterpillars, found the same anastomosing of the tracheal capillaries. Emery, however, states that in *Luciola* the tracheoles always end free.

Max Schultze, in 1864, in studying osmic acid preparations of *Lampyris splendidula,* found at the periphery of the cylinder certain darkened cell-like bodies into which the smaller tracheal twigs entered. Failing to find any continuation of the tracheae beyond these bodies, he called them the "tracheal end-cells".

In osmic acid preparations of *Photinus* there is always a blackening of the smaller tracheae at the point of division to form the tracheoles; the tracheoles themselves are blackened throughout their extent. Surrounding the point of origin of the tracheoles the tissue is also more or less blackened. In the typical preparations with this fixation there are darkened rounded bodies which might seem at first to be cells. In other preparations there are irregular masses instead of cell-like bodies. The dark cell-like bodies are found in *Luciola,* where the structure closely resembles that of *Photinus.* As these bodies are only found in osmic acid preparations, Emery regarded them as an artifact and not as true cells. In *Photinus* they occur only in osmic acid material, vary considerably in different material, and are always accompanied by an apparent shrinking and distortion of the tissue of the entire cylinder. It would seem probable, therefore, that they are an artifact. In any case they are incorrectly termed "end-cells", as the tracheoles extend to a considerable length beyond them.

Wielowiejski and Bongardt have found the "end-cells" described by Max Schultze in *Lampyris splendidula,* but have also found that they do not contain the ultimate tracheal endings, the tracheoles passing out of them. In *Lampyris,* it is asserted, the "end-cells" are formed by the epithelium of the tracheoles. In the "tracheal capillary end network" found by Wistinghausen and Holmgren in Lepidopterous larvae, it is stated that the epithelium is spread out in a web-like manner to form the "end-cells". Wielowiejski, Bongardt, Holmgren, and Wistinghausen thus all agree that the tracheal epithelium is abnormally developed and spread out to form the "end-cells", or "transition cells" as Holmgren more correctly terms them. Emery considers the cylinders of *Luciola* as homologous to the "end-cells" of *Lampyris,* and as modified tracheal epithelium.

The cylinders in *Photinus* are clearly shown to be of cellular structure. They contain numerous small nuclei near the tracheal axis, but clearly separate from the tracheal epithelium, which may be traced even to the smaller branches. Throughout the photogenic tissue of *Photinus* the tracheal epithelium is very thin, with large flattened nuclei, and there is no indication of abnormal development. Another striking difference from the conditions in *Lampyris splendidula* is that in the latter Bongardt found the "end-cells" and tracheoles more abundant in the dorsal layer than in the ventral. In *Photinus* these structures are clearly confined to the ventral layer.

As to the derivation of the photogenic tissue, the form and content of the cells has led to a general idea that it is modified fat body. Raphael Dubois, from a study of *Lampyris noctiluca* and *Pyrophoms noctilucus*, claims that it arises by proliferation of the cells of the hypodermis. His statements are based on a study of the developing organs from the ovum to the adult insect. He further finds that in the development of the organs by means of the physiological changes incident to photogeny, the dorsal layer becomes filled with opaque granules and ceases to be photogenic. The marked difference in structure between the two layers in *Photinus* make it difficult to believe that in the adult insect, at least, there is any growth of one at the expense of the other. There is no apparent difference in the relative thickness of the two layers at the beginning and at the end of the flying season.

Summing up the structure briefly, we find that there are two distinct layers. The ventral layer is made up of two elements, the cylinders with their tracheae and the parenchyma. Throughout the parenchyma is a network of fine air capillaries.

When a fresh light organ is studied under a microscope, the dorsal layer is found to be non-photogenic, opaque, and chalky white. The ventral layer is seen to be the truly photogenic layer. When the ventral surface is studied under a microscope in the dark-room, the light is found to be uniformly distributed through the parenchyma, the area of the tracheolar network. The cylinders stand out against this luminous background as non-photogenic rings. It is thus evident that the process of photogeny takes place in that portion of the ventral layer which lies between the cylinders, and it is exactly this portion of the tissue which has a provision of air not known elsewhere in insect histology.

The light of the fire-fly is entirely independent of the life of the photogenic cells. When the light organs are crushed the light becomes more brilliant as the tissue comes more directly in contact with the air and continues for several hours if the tissue is kept moist. Crushed light organs become brilliantly photogenic under the influence of oxygen, and the light continues for several hours. If slides with crushed organs are placed in a jar of CO_2 the light is quickly extinguished, but when the slides are transferred to a jar of oxygen the light reappears instantaneously.

With the exception of Raphael Dubois, all modern workers on organic light agree that it is a process of oxidation. In the fire-fly it is supposed to be the oxidation of some substance secreted by the parenchyma cells of the ventral layer. The exact nature of this substance has not yet been determined. Watosé states that it is of a fatty nature. Radziszewski, through his study of the artificial luminosity of lophin, discovered a series of carbon compounds similar to those found in living organisms and capable of becoming luminous under conditions compatible with life. These conditions he found to be slow chemical action in the presence of alkalies. He found that the caustic alkalies could be replaced by cholin, neurin, and other substances found in living creatures. The light from these carbon compounds was shown by the spectroscope to be identical in nature with that produced by photogenic organisms. Watosé had previously stated that the light of the fire-fly was the result of oxidation in alkaline media of a granular secretion of the photogenic cells.

The only opposing voice is that of Dr. Raphael Dubois. He wholly discards the oxidation theory and substitutes for it that of a reaction between two substances which he claims to have isolated and to which he has given the names luciferose and luciferine. He admits that oxygen and moisture are essential to the reaction. He states, however, that in his experiments, the effect of oxygen was to extinguish the light without first giving any increase in brilliancy.

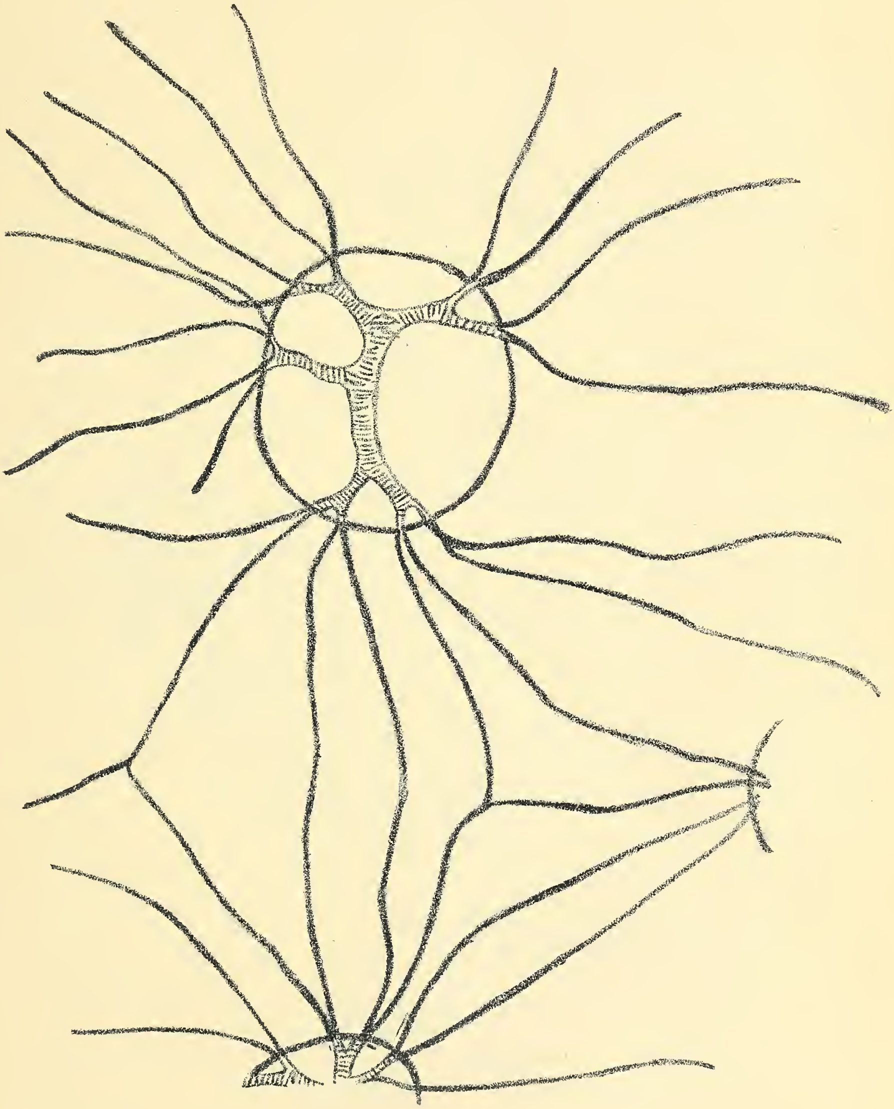
As a large part of Dubois' experiments were upon a marine mollusc, it seemed that a comparative study of the light of *Photinus* with that of some marine organism would be helpful. Consequently I followed the work previously done with the fire-fly by a summer at the Marine Biological Laboratory at Wood's Hall, Massachusetts. The form chosen was *Mnemiopsis leidyi.* The work done was very far from complete, but even so it seems significant.

Mnemiopsis leidyi, one of the Ctenophores, is quite common near Wood's Hall during the summer months. It is a beautiful jelly-fish, almost as transparent as the sea water. The specimens upon which I worked varied from one to six inches in length. The light is produced by cells lying between the longitudinal rows of swimming plates. It is given only when the Ctenophore is disturbed in some way.

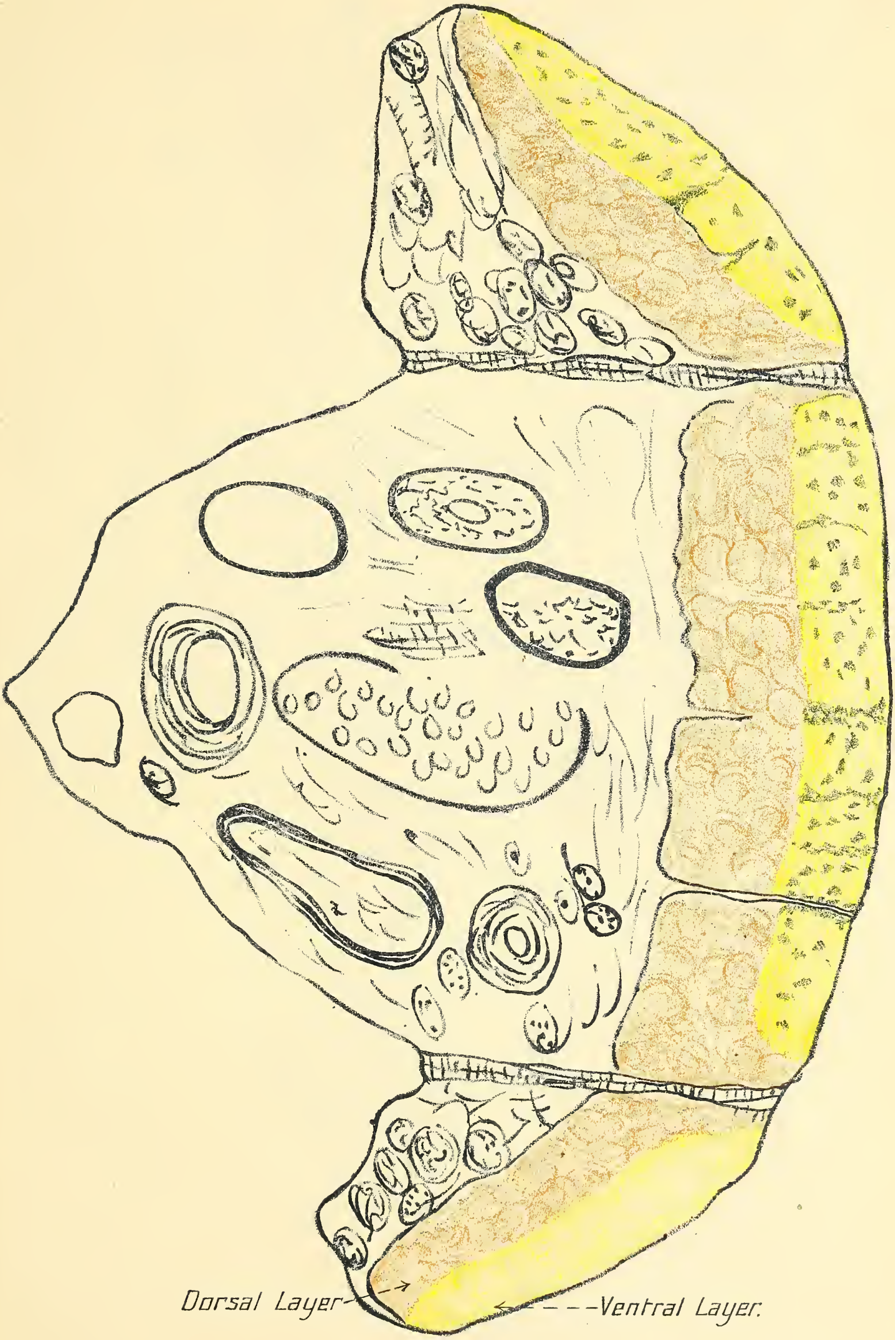
When the living animal is placed in various solutions and gases the effect seems to depend almost entirely upon the extent to which it is irritated. The same agents give entirely different results when the photogenic tissue is brought directly under their influence. When live Ctenophores are placed in acid and alkaline solutions they flash more frequently in the former, but very soon die in both. When the photogenic tissue itself is placed in acid solutions, the light is wholly extinguished and does not reappear under the influence of oxygen. In alkaline solutions the light persists for a considerable time and becomes more brilliant when oxygen is passed over the tissue. Material left for half an hour in an acid became luminescent when an excess of alkali was added, and gave a brilliant light under the influence of oxygen.

There remains a wide field for investigation. There are very great differences in structure between fire-flies of closely related genera, and little has yet been done with the multitude of marine light-givers. I believe, however, that in all forms, however widely separated, we shall find the process of photogeny to be essentially the same and to be one of oxidation.

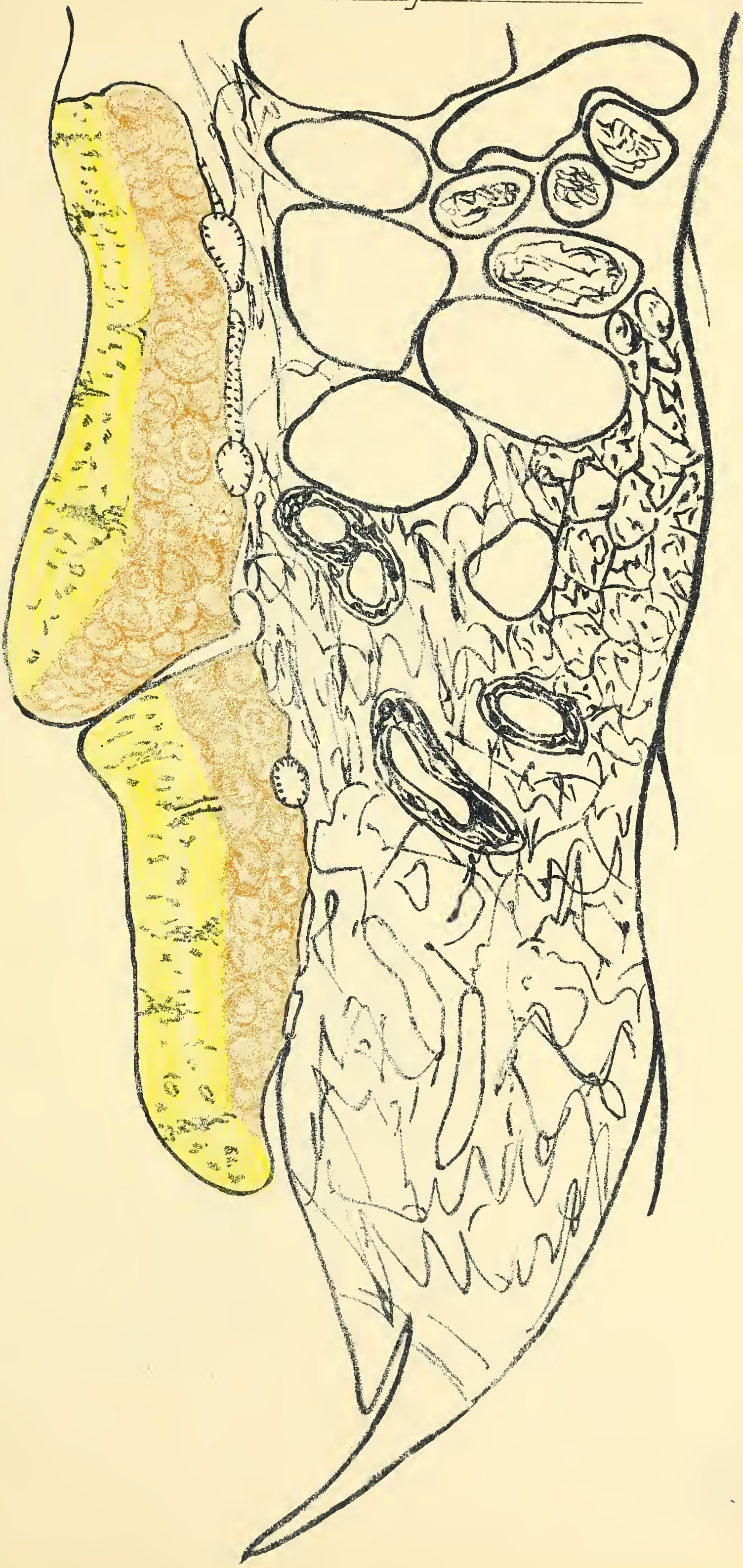
Teazed preparation to show anastomosing of Tracheoles.



Transverse Section through Abdomen.



Longitudinal section of Abdomen From 5th Segment to Caudal End.



Typical osmic Acid preparation.